REGENERATION, VARIATION AND CORRELATION IN THYONE

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It is well known that many Echinoderms possess a remarkable power of regeneration, and the results given here show some interesting phases of this process in *Thyone briareus* (Leseur). The problem was suggested a few years ago in connection with class work in the Marine Biological Laboratory at Woods Hole, Massachusetts. There it is a common practise for students who are taking the invertebrate course to keep aquaria in which are placed specimens brought in from various collecting trips in the vicinity. Students are encouraged to study the behavior of these animals, but their enthusiasm for collecting frequently causes them to overcrowd their aquaria, with disastrous results. After collecting Thyone, especially if they are kept in stagnant water, the student is frequently amazed to find one or more of his specimens that have undergone evisceration. In this process the animal not only loses the principal feeding organs, the tentacles, and the entire digestive system, consisting of the esophagus, stomach and intestine; but it also throws out a whole series of organs surrounding the esophagus including the circlet of calcareous plates, the nerve ring forming the central nervous system, the portion of the water-vascular system known as the ring canal with its attached stone canal and Polian vesicles, and the muscles which serve as retractors for the set of organs surrounding and attached to the esophagus. We shall refer to these muscles as retractors of the esophagus.

The remainder of the animal after evisceration consists, principally, of the dermo-muscular integument, the

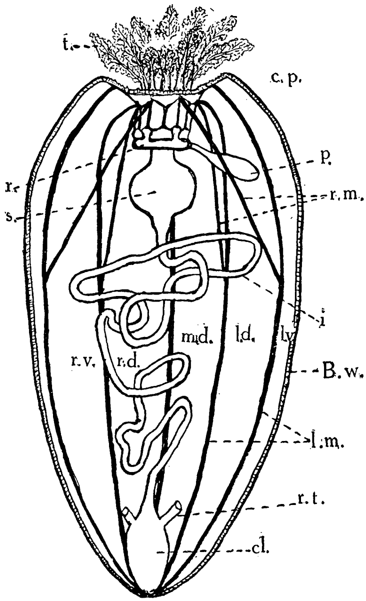
cloaca with its attached respiratory trees, the single gonad, the radial canals of the water-vascular system and the major portion of the dorsal mesentery by which the intestine was suspended. Since this part of the animal continues to give reactions, the student invariably raises the question, "Can Thyone regenerate the lost parts?" This question was the starting point of the following investigation. The work had not proceeded far when it was discovered that important individual differences occurred, and the question became, "To what extent, or how completely, may these individual variations be reproduced in the process of regeneration?" Curiously enough, the most important differences between individual Thyone involve structures which help to form the radial symmetry of the animal. Consequently the problem has a bearing on the phylogeny as well as the ontogeny of Thyone.

In general, the results show that regeneration of all lost organs may occur and that there is a decided tendency to even reproduce individual variations. It was found that the Polian vesicles varied greatly in number, size and location. The retractor muscles in a single radius were single or multiple, and for each individual this variation was closely correlated with a corresponding variation in the number of Polian vesicles. Whether one or more Polian vesicles are present, there is a strong tendency for these to occur on the left side of the animal, a fact which undoubtedly has a phylogenetic significance. A more complete statement and a discussion of these results will be given in the following pages.

General Structure of Thyone

Thyone is functionally a bilateral animal. It has anterior and posterior ends, dorsal and ventral surfaces, and consequently right and left sides. The external opening of the genital duct is located near the anterior end in the mid-dorsal region. The structure and arrangement of the tentacles is alike on both sides of the animal. Even

the feeding reactions, as Pearse has pointed out, indicate a bilateral type. The single genital gland is median in position; the genital duct and the stone canal are in the median dorsal mesentery; a part of the intestine and the stomach are supported by the same structure. The respiratory apparatus is also a bilateral structure, one branch arising from each side of the cloaca.



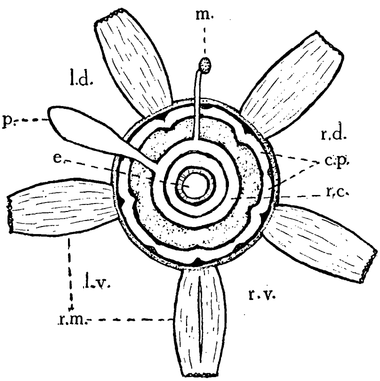


FIG. 1. FIG. 2.

FIG. 1. A DIAGRAMMATIC DRAWING FROM A DISSECTION MADE BY TAKING A LONGITUDINAL CUT IN THE BODY WALL A LITTLE TO THE LEFT OF THE MID-VENTRAL LINE. Shows the arrangement of the chief organs concerned in evisceration and subsequent regeneration. *B, w.*, body wall; *cl.*, cloaca; *c. p.*, calcareous plates; *i.*, intestine; *l.* m., longitudinal muscles; *p.*, Polian vesicles; *r.*, ring canal; *r. m.*, retractor muscles; *r. t.*, base of respiratory tree; *s.*, stomach; *t.*, tentacles; *m. d.*, mid-dorsal; *l. d.*, left dorsal; *l. v.*, left ventral; *r. d.*, right dorsal, and *r. v.*, right ventral, interradial spaces.

FIG. 2. A DIAGRAM TO SHOW THE RELATION OF RADIAL TO BILATERAL SYMMETRY. The esophagus (*e*) is shown in cross-section, cut just anterior to the stomach, and the view looks toward the anterior end. *M.*, madreporite; *r. c.*, ring canal. Other letters as in Fig. 1.

Notwithstanding this general tendency toward bilateral symmetry, the most conspicuous differences between individuals involve structures of the radial type. Fig. 1 is a diagrammatic drawing of a dissection to show the general

arrangement of some of the more important structures studied in this experiment. The dissection was made by making a longitudinal cut in the body wall a little to the left of the mid-ventral line, and then pulling the flaps apart and pinning the animal down on its dorsal surface. The Polian vesicle is shown attached to the ring canal in the position where it is usually found when only one is present, that is in the left dorsal interradial space. It will be noticed that the retractor muscles are simply branches of the longitudinal muscles, and hence are radial in position. At the time of evisceration the body wall breaks a short distance posterior to the tentacles, the retractor muscles separate at the point where they join the longitudinal muscles and the intestine breaks off just in front of the cloaca.

A better understanding of the radial type of structure will be gained by a reference to Fig. 2. This figure is a diagram to show the relation of the radial to the bilateral symmetry. The dorsal side of the animal is represented toward the top of the page, the esophagus appears in cross-section, cut just anterior to the stomach, and therefore one is looking forward to the other organs shown. The retractor muscles, showing the position of the radii, are much contracted and thickened, a condition in which they are usually found after evisceration. The stone canal ending in the small madreporite is located in the mid-dorsal interradial space. Passing around in a clockwise direction, the other interradial spaces are designated as right dorsal, right ventral, left ventral and left dorsal. Polian vesicles may be found in any of the interradii except the mid-dorsal space which always bears the stone canal. Although only one Polian vesicle is represented in this figure, the mid-ventral retractor muscle is shown double, a split condition which is characteristic when two or more Polian vesicles are present. This description will be sufficient to show the general relation between the radial and the bilateral symmetry.

Evisceration

Only one method of producing evisceration was used. By placing a number of Thyone in a small aquarium of stagnant sea water, the supply of oxygen is soon exhausted. The animals become greatly distended, they crawl up on the sides of the aquarium when possible, and extend the siphon toward and frequently above the surface of the water. All of their behavior, including the pumping of the siphon, indicates that respiration is inadequate. In the course of a day or two the water becomes very foul; soon some of the Thyone will eviscerate, and a considerable percentage will do so as conditions grow more unfavorable. Many, however, resist the unfavorable surroundings and will not eviscerate though kept for several days in foul water. But if the aquarium is now placed where it will have a continuous stream of water and air bubbles passing through it, the behavior of the animals is somewhat different. They then tend to contract to a minimal size, and sometimes assume a volume not more than one fifth to one seventh of their maximum distention. The respiratory movements are practically discontinued; the animal seeks a position as close as possible to the side and bottom of the aquarium. Contraction does not always take place immediately. To my surprise, after several hours I found Thyone which had resisted the previous unfavorable conditions now discharging their viscera. After remaining two or three days in the running water, and the animals had apparently become adjusted to this condition, I again set the aquarium to one side partly filled with water. Then, by repeating the conditions of the first experiment, as the water became foul several more of the holothurians apparently found life too strenuous to further retain their internal organs. When the remainder of this lot of Thyone was returned to running water, and again to stagnant water, a few additional individuals underwent self-mutilation. Out of a total of sixty-one specimens used in this lot forty of them eviscerated. That is, autot-

omy occurred in at least sixty-five per cent. of Thyone, under the conditions described. Probably one reason why this process did not occur in a still larger number is that some animals occupied more favorable positions in the aquarium. A discussion of the cause of evisceration will be given later.

When evisceration occurs it is sometimes hard to see just how the process takes place. Pearse ('09) ascribes the process to a "structural accident"; that is, it is due to a powerful contraction of the circular muscles at a time when the calcareous ring is well forward. "But if the tentacles are extended," he says, "and the calcareous ring is pushed forward a break may occur at *b*" (a point in his Fig. 2 where the body wall joins the calcareous ring) "as a result of the strong contraction of the circular muscles at that point, and the visceral organs are forced out. . . . Whether this autotomy takes place or not depends upon the breaking of the inner branch of the longitudinal muscle bands, whose normal function is to retract the calcareous ring. When the strain brought about by the contraction of the circular muscles becomes too great these inner bands are torn asunder, usually at the point *x*" (inner end of the retractors of the calcareous ring). While it is true that muscular contraction and consequent pressure undoubtedly plays a prominent part in the process, close observation has convinced me that this is not the only factor causing evisceration. Upon several occasions I have watched carefully the breaking of the body wall near its attachment to the calcareous ring, and while there are times when the pressure appears to be strong, especially when the animal is being irritated mechanically, there are other times when the skin appears to "melt away" or separate with very little or no pressure present. Indeed, after the skin once breaks at one side and the viscera escape through the opening, the pressure is relieved. But one may observe that the skin continues to break until the calcareous ring is entirely separated. This, of course, would not happen if the process

depended entirely upon an accidental structural defect. Another thing noticed is of interest in this connection. When splitting open the body wall of an animal that was eviscerating, and thus relieving any internal pressure that might be due to contraction of the circular muscles, some of the retractors were seen still attached to the longitudinal muscles. Under these conditions it would not be possible for the retractors to exert any pull against the pressure produced by the circular muscles, yet the retractors were observed to constrict off or break away from the longitudinal muscles by what appeared to be purely a local disturbance. It is hard to see how this could happen, or how the skin continues to separate around the calcareous ring after the first break is made, if the process of evisceration depends solely upon the breaking of retractors and internal pressure. Indeed, the view that local changes take place in the tissues is supported by other facts. Leptosynapta, if left in stagnant water or under other favorable conditions, undergoes repeated autotomous fission as the result of local constrictions, and Pearse states that autotomy depends upon the presence of the anterior portion of the body, and presumably upon the presence of the circumoral nerve ring. However, he found in Thyone that highly irritating substances like acetic acid and clove oil did not produce ejection of the viscera.

> Nor were drugs like codene and atropine, which cause violent peristaltic waves of contraction to pass over the body, any more potent in inducing autotomy. The same may be said of sodium chloride, atropine and clove oil, although the injection of any of these substances was often followed by a waving of the oral tentacles to perform feeding movements, thus bringing about favorable anatomical relations for autotomy.

These results would indicate that the nervous system is not primarily involved. Certainly the ejection of viscera may occur in Thyone without any visible external stimulus.

The parts eviscerated in Thyone have already been

mentioned. However, sometimes evisceration is incomplete, as the following examples will show. On the morning of August 4, a Thyone, which we shall later speak of as individual *H*, was found eviscerating in an overcrowded aquarium jar. While the process usually requires only a few seconds, or at most a few minutes, the intestine in this case was not completely thrown out until two or three hours later. This animal lived until killed at the end of twenty-one days. In the afternoon of the same day on which individual *H* eviscerated, another Thyone was found with the process only partially complete. Five hours later the intestine was still retained, and scissors were used to cut it off at its anterior end near the stomach. Though this Thyone received equally good care it died at the end of two days without further evisceration. A third specimen was found incompletely eviscerated on the above date, but it was allowed to stand until the next morning; at this time the injured end was open, the intestine was still within the body cavity and a part of one of the branchial trees was protruding. The intestine was pulled out and broken off, after which the branchial tree was retracted and the injured end partially closed. This animal also died at the end of two days. A fourth Thyone was seized and by squeezing was forcibly caused to throw off the usual parts except the following: a part of the stomach, most of the intestines, and some of the retractor muscles which had broken off near their esophageal end. The next morning it had expelled the remainder of the stomach and intestine, two complete retractor muscles, and some débris which had escaped from the intestine into the body cavity. The anterior end of the part remaining appeared ragged and imperfectly closed. It died on the third day. It is probable that the two retractor muscles last expelled were broken off at their posterior ends by local constriction, not when the body was under pressure. A fifth animal, which we shall designate as individual *M*, was found partly eviscerated

late on the afternoon of August 6. The next day it still retained the stomach and intestine and at noon the digestive tube was clipped off with scissors in the region of the esophagus. Nothing peculiar was noted in its behavior until four days later, August 11, when it discharged the remainder of the digestive tube. It lived and was killed at the end of eighteen days. These results are typical. The animal dies unless it is itself able to eliminate all organs concerned in the process of evisceration, and therefore regeneration does not occur unless all these organs are eliminated.

The eviscerated animals show comparatively a low degree of mortality. In an attempt to raise twenty-five mutilated Thyone seven died; three of these were unable to complete the process of evisceration as described above, and two more, since they lived for fourteen days, probably owe their death to other causes. The sixth specimen to die lived three days and had been slow in eviscerating. The seventh did not receive the best of care and died after three days. So considering the amount of injury the mortality is extremely small where proper care is taken and evisceration is complete.

It will not be inopportune to describe the subsequent behavior of the different parts after evisceration. The parts expelled lie on the bottom in a more or less inactive condition until they die, which happens usually in the course of a few hours. At first the tentacles frequently expand and contract. They are highly sensitive, as one would expect, and if touched withdraw quickly into the esophagus and at the same time the retractor muscles will undergo strong contraction. By supporting these parts near the surface of the water, so as to insure plenty of oxygen, an attempt was made to keep them alive. In some cases the parts remained alive for two or three days, so this experiment appeared to be partially successful. Death is probably due to the direct exposure of tissues to the sea water and to the attacks of minute organisms. The dermo-muscular portion of Thyone is

much less sensitive than the expelled portion, just after evisceration. This is due to lack of a central nervous system.

Behavior During Regeneration

After evisceration each specimen was placed in a separate jar of fresh sea water. The injured end of the body turns in and closes up tightly, and the entire body is somewhat smaller than before evisceration. Respiration is slower and not so vigorous. If the water is stagnant, within a few hours the animal usually climbs up on the side of the aquarium by means of its tube feet. This part of the animal therefore is capable of responding to a lack of oxygen, and the reaction is independent of the central nervous system.

The observations upon the following individual, referred to in my notes as Thyone *A*, will serve to illustrate the general behavior during regeneration:

July 14, A.M.—Animal eviscerated itself in the usual way. In the afternoon it climbed up on the side of the jar and clung there evidently for the purpose of respiration.

July 15–16.—Acts as on the afternoon of the fourteenth. Keeps closed and well contracted at the injured end. Entire body somewhat smaller than before evisceration, due in part to organs lost. Respiration slower and not so vigorous as normal.

July 17.—In the afternoon, after water was changed, Thyone took up position on the sand against the side of the jar farthest away from the source of light.

July 18.—The next morning it was half buried in the sand in same position, with a few pieces of débris pulled over it. Remained so all day.

July 23.—For some two days it has been slowly burrowing down until only the two protruding ends of the body can be seen. When a piece of débris that was being held over a part of the anterior end was touched, this end retracted below the surface and the posterior end withdrew until it could scarcely be seen. Later the posterior end retracted when the shadow of my hand passed over it, the hand being held about one foot away. The uninjured animal is even more sensitive to shadow. The respiratory movements are growing stronger.

July 28.—For the past two or three days the Thyone has been slowly moving through the sand in a posterior direction without uncovering itself.

August 2.—It is now oriented with respect to the direction of the light and has reached probably the darkest portion of the jar.

August 7.—Has advanced still farther. Came about half way out of the sand to do this.

August 8.—Reacts quickly to shadows by withdrawing, and to jarring the table. Evidently is recovering its normal behavior.

August 10.—Has again come up about half way out of the sand. Reacts quickly to shadows as before.

August 11.—Came entirely out of the sand. Spent the day on the sand or on the side of the jar. Appeared restless.

August 12, 4 P.M.—Has been clinging to the side of the jar and moving about more or less all day. Respiratory movements are strong and apparently normal. Has just now expanded the anterior end sufficiently for me to see the new growth of tissue formed around a pentagonal opening. Fifteen minutes later it was observed to extend a set of minute tentacles and go through feeding movements. The tentacles appeared to be slightly more than three eighths of an inch in length. Its behavior continued apparently normal until it was killed twelve days later.

The actions of other Thyone were studied under the same conditions, and we shall now give a general summary of their behavior during regeneration. The earliest reactions after evisceration take the form of contractions resulting in the closure of the wound, and movements in response to lack of oxygen. If the oxygen supply is sufficient Thyone will draw itself closely into the angle between the side and bottom of the aquarium, or if the supply is deficient, it clings close to the side of the jar near the surface. In from three to seven days an instinct to burrow usually asserts itself. There is a tendency for the body to contract very noticeably at this time, and the whole organism becomes rather inactive. This condition is probably necessary for the formation of new tissue. Pearse makes the statement that in burrowing the normal Thyone will cover itself in from two to four hours. My observations on the mutilated animals indicate that they require from twelve to twenty-four hours, in one case forty-eight hours, to complete the reaction. The process frequently stops for some hours and occasionally is never completed. In the Thyone de-

scribed above the animal did not begin to orient itself with respect to the source of light until about the twelfth day, but in another case the response took place on the second day, which shows that this reaction does not depend upon the central nervous system. It should be stated that normal Thyone similarly placed were used as controls. Thyone *A* was quite sensitive to shadows and to touch on the ninth day, but it reacted more quickly on the twenty-fourth day both to shadows and to mechanical disturbances. Whether this was due to the regeneration of a new central nervous system, or to a more highly developed specialization of function in the old tissue, I am unable to say. It is quite possible that both factors were involved. Respiration is undoubtedly correlated with the activity of the animal, and feeding movements do not occur until the regeneration of all organs is well established, at about twenty-seven or twenty-eight days.

The internal changes that take place during regeneration were studied in animals that were killed at different stages in the process. Thyone *N* was killed nine days after self mutilation. At the injured end there was a very small plug of tissue representing the newly formed esophagus; a thread-like continuation of this tissue, the beginning of a new stomach-intestine, was also seen in the mesentery. The calcareous ring and the ring canal were not clearly defined. Another Thyone was killed at about the same age after evisceration; India ink was injected into the cloaca and into the opening at the anterior end in an attempt to demonstrate a cavity in the newly formed thread-like, stomach-intestine. The results were negative and the esophagus was found to be tightly closed. However, the interesting observation was made that the anterior end of *each of the longitudinal muscles had split off a very slender branch to form a new retractor muscle* (see Fig. 3). These newly formed retractor muscles were not more than one-fourth inch in length; their anterior ends were attached in a normal position around the esophagus, but their posterior ends

were attached only a short way back, much in front of the position of attachment of the full-sized retractors. In another animal killed when a day or so older, the same conditions held with reference to esophagus, stomach and intestine. At least three of the radial canals belonging to the water vascular system had branched and connected at their anterior ends in such a manner as to form a part of a new ring canal (cf. Fig. 4). I was unable to find the rest of the ring-canal and perhaps it was not yet complete.

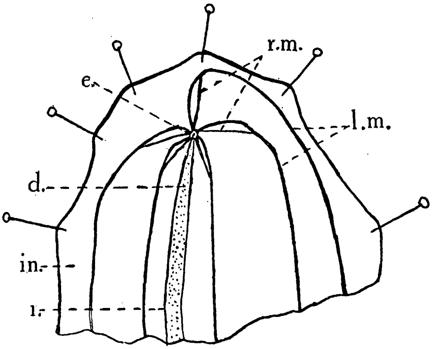


Fig. 3.

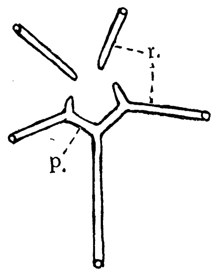


Fig. 4.

Fig. 3. Diagrammatic Drawing to Show that in Regeneration the Retractor Muscles (*r. m.*) Arise by Splitting off from the Longitudinal Muscles (*l. m.*). Dissected a little to the right of the mid-ventral line; *d.*, dorsal mesentery suspending the intestine (*i.*); *in.*, integument; *e.*, region of esophagus.

Fig. 4. To Show the Development of the Pentagonal Canal in a Thyone about Nine or Ten Days after Evisceration. *r.*, radial canal; *p.*, pentagonal canal. The anterior ends of the radial canals fork dichotomously, and these branches anastomose to form the canal which later assumes a circular shape around the esophagus.

Thyone *F*, which was killed twelve days after evisceration, showed minute calcareous plates which formed a very small esophageal ring not more than one millimeter in diameter. The esophagus continued posteriorly in the form of a small tube, the stomach-intestine, which was suspended in the dorsal mesentery. This new digestive tube was about 0.5 millimeter in diameter and contained small, colored, movable particles that could be seen with the unaided eye. The ring canal was completely formed.

Another specimen, Thyone *O*, died at the end of four-

teen days and was in bad condition when examined. The stomach had begun to expand and retractor muscles were present. Probably owing to the condition of the specimen, no calcareous ring, ring canal, or Polian vesicle could be found. Another individual killed at about fifteen days showed the stomach slightly enlarged, and the intestine, retractor muscles, calcareous ring, tentacular canals, and ring canal well formed. Two small Polian vescicles each about one millimeter in length were present. The position of the new intestine was described in my notes as follows:

From the stomach the intestine follows the ventral edge of the dorsal mesentery, lying ventral to the gonaduct. At the gonad it turned ventrally with the mesentery and then forward for about one half inch to the left interradial space; here it turns rather abruptly backward, continuing in the mesentery below the left branchial tree to the anterior ventral part of the cloaca.

At a little later stage in another specimen the intestine passed from the left ventral interradial to the right ventral interradial space; then posteriorly and again to the left, following the ventral radial mesentery to the anterior ventral side of the cloaca.

We see from the preceding description that all important organs have been reproduced in form though not in size, before the end of the fifteenth day. The first madreporite with its tiny stone canal was found some eighteen days after mutilation. Twenty-one days after evisceration in one specimen the calcareous ring was about three millimeters in diameter and the ampullæ at the bases of the tentacles were well developed. Within a week after this time the regenerating animal begins active feeding. Thyone *A,* killed at 41 days, was practically a normal animal both in behavior and appearance, except for the fact that the regenerated organs had not yet reached full size. The stomach was about one third normal size, but the Polian vescicles were better developed. The intestine contained a small amount of food material and was nine or ten inches in length; most of this growth had

taken place posterior to the gonad. It was held in position as previously described and had several additional coils.

INDIVIDUAL VARIATIONS

To all outward appearances any two Thyone are as much alike as two peas. It was not until the internal organs were studied that important differences were ob-

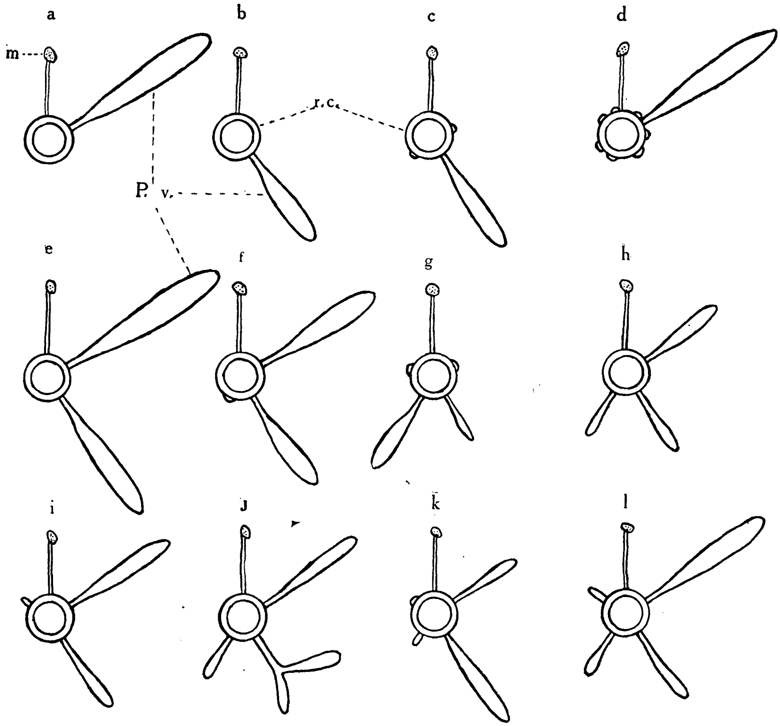


FIG. 5. DIAGRAMS TO SHOW VARIATION IN POSITION AND SIZE OF THE POLIAN VESICLES. *P. v.*, Polian vesicles; *m.*, madreporite; *r. c.*, ring canal; *a-d*, with one Polian vesicle, *e-g*, with two; *h-k*, with three, *l.*, with four; *c.*, *d.*, *f.*, *g.*, *k.*, with additional rudiments of these vesicles; *j.*, with a branched vesicle.

served. While there are numerous minor differences, the most conspicuous variations are found in the number, size and location of the Polian vesicles (cf. Fig. 5), and in the number and arrangement of the retractor muscles. On account of the radial structure of Thyone not more than four Polian vesicles are present, since

a homologous structure, the madreporite and its stone canal, occupies the dorsal interradial space. The number of vesicles varies in fact from one to four. By a reference to Table I, it will be seen that out of 77 individually examined, 41 had one, 20 had two, 14 had three,

TABLE I

To Show the Number of Polian Vesicles Present in a Given Number of Thyone. Also to Show their Location in the Interradial Spaces, with Reference to the Bilateral Symmetry of the Animal

Number of Polian Vesicles	Number of Individuals Examined	Left Dorsal	Left Ventral	Right Ventral	Right Dorsal
1	41	38	3	0	0
2	20	17	19	3	1
3	14	14	15	12	1
4	2	2	2	2	2
Totals..........	77	71	39	17	4

and 2 had four Polian bodies. If one is to test the matter of regeneration, of course it is important to know whether the variations or individual peculiarities will be accurately reproduced. Another striking characteristic comes out when we note in the same table the location of these organs. Of the forty-one individuals which had a *single Polian vesicle, all were on the left side of the animal,* and 38 were in the left dorsal interradial space. In twenty specimens with two Polian bodies each, 36 were on the left side and only four on the right side of the body. A similar asymmetrical distribution of these parts was found when three Polian bodies were present. In one specimen, however, two vescicles were found in one space, the left ventral interradius, the only instance of this kind observed; on account of this doubling, the right side lacked one of the number to which it was entitled in the table. Where four Polian bodies are present the arrangement is, of course, symmetrical on both sides. Still another interesting fact comes out when we examine the totals in the last line. Out of the 77 individuals, 71 had a Polian vesicle in the left dorsal interradial space, 39 vesicles were found in the left ventral,

17 in the right ventral, and only 4 in the right dorsal space. That is, the total number on the left side compared with the total number on the right side bears the ratio of 110 to 21. Not only is there this tendency for the vesicles to be more abundant on the left side of Thyone, but the totals show that *the chances of a given Thyone having a Polian vesicle in any given interradial space decreases in a counter-clockwise direction,* beginning with the left dorsal interradial position. Coinciding with the number of individuals examined, the maximum number of chances is found in the mid-dorsal interradius, where the stone canal is always present. That is, the stone canal with its madreporite is a more fundamental and stable structure than each or all of the vesicles.

The conditions are none the less interesting when we compare the Polian vesicles with reference to size and location, as will be seen from the examination of Table II. The Polian vesicles are here divided arbitrarily into three groups, designated as large, medium and small, and their respective locations are shown. In addition some Thyone had the rudiments of other vescicles, each too small to be considered a distinct pouch. These are designated in the table as a "rudiment." It will be noticed that all of the large, and most of the medium-sized vesicles are on the left side; that all the small ones, and most of the rudimentary ones are on the right side. The table as a whole shows that not only does the number of Polian vesicles diminish in a counter-clockwise

TABLE II

TO SHOW THE POLIAN VESICLES WITH REFERENCE TO SIZE AND LOCATION

Size	Left Dorsal	Left Ventral	Right Ventral	Right Dorsal	Total
Large.......	56	17	0	0	73
Medium.....	17	22	5	1	45
Small.......	0	0	10	3	13
Rudiment...	2	1	5	7	15
Total.......	75	40	20	11	146

direction, but their size diminishes following the same law. These facts appear significant and without doubt are suggestive of ancestral history.

If it is true that the radial symmetry of Echinoderms is to be ascribed to a fixed stage in their ancestral history, we are led to suppose that the point of attachment was on the right side of an originally bilateral animal. The life history of Pentacrinus, the larval organ of Asteroidea, and a great many anatomical and embryological facts support this view. While it is not within the province of this paper to discuss the relative significance of these matters, the evidence is so overwhelming that the theory is generally accepted. It is also no doubt true that some groups of Echinoderms took to a free-living existence early in their ancestral history, and others remained fixed until comparatively a late period. As proof we may cite the embryological evidence that Holothurians develop without any attached stage whatever, that the Asteroids develop a larval organ and pass through a Sessile stage for a brief period in their development, while the crinoids usually remain permanently fixed throughout life. At least we can best account on this theory for the deep-seated and fundamental radial symmetry of some forms; the longer the attachment the more deep-seated would become the type of radial symmetry. Now if this theory is correct we can use it to explain the conditions described above for Thyone. The ancestors of this form must have broken away from the fixed stage very early, for we find the radial symmetry not well established on the right side of the animal as evidenced by both the position and size of the Polian vesicles. Out of 118 large and medium-sized Polian vesicles, 112 were on the left side, while in a total of 28 small or rudimentary Polian bodies, 25 were found on the right side. The arrangement of these organs in Thyone adds one more bit of evidence to support the following statement of Lankester.

It therefore appears that the Holothurian stock branched off from the Pelmatozoa before complete pentamerous symmetry of the hydrocoele and associated organs had arisen, before any definite calcynal system had developed, while the gonads were still a simple strand opening to the exterior by a single posterior gonopore.

The muscles used as retractors of the œsophagus were other organs in which there was considerable individual variation. As a general rule each of the five retractor muscles consists of a single band that takes its origin from the longitudinal radial muscle about one third the way back from the anterior end of the body and is inserted in front into the wall of the esophageal ring. Such a retractor, however, is frequently split up into several strands varying from two to five in number. A reference to Table III

TABLE III

To Show the Correlation between the Number of Polian Vesicles and the Tendency for the Retractor Muscles to Divide

Number of Polian Vesicles	1	2	3	4
Retractor muscles, single	39	2	0	0
Retractor muscles, multiple	1	17	15	2
Average number retractor muscles, per individual	5.153	10.263	12.400	10.000
Average number retractor muscles, per radius	1.030	2.052	2.480	2.000

shows that in 76 individuals examined, 41 had retractor muscles all in single bands, while 35 specimens had these muscles subdivided or multiple in character. This variation is especially interesting when considered with reference to the number of Polian vesicles. For in forty cases where one Polian body was present thirty-nine bore the unsplit or single retractor and there was only one specimen with these muscles showing a multiple number. In thirty-six cases where two or more Polian vesicles were present, all but two had the retractor muscles in a split or divided condition. If we consider each strand as a separate retractor muscle, we may then obtain the average number of retractors per individual for any definite number of Polian vesicles. By a reference to the fourth horizontal line of Table III, one finds that the average number in individuals with one Polian vesicle is

just slightly in excess of five, the pentameric number, and the average number when two Polian vesicles are present is 10.263. This ratio is only partly maintained when three vesicles are present, for the average number is then 12.400, and in the two cases with four vesicles the average was just twice the pentameric number. It is therefore evident from the facts shown in this table that with an increase in the number of Polian vesicles there is associated a strong tendency for the retractor muscles to take on a split character. If it were not for the fact that the split character shows considerable variation in the same individual one might suggest that the tendency to divide is correlated with the greater functional activity of the water vascular system as evidenced by the increased number of Polian vesicles and the location of the longitudinal muscles that lie along and just internal to the radial canals. About all one can say is that correlated with a more complete radial symmetry with respect to the Polian vesicles, there is a greater plasticity in the retractor muscles, causing them to divide longitudinally into separate muscle bands.

To what extent, or how completely, may these individual variations be reproduced in the process of regeneration? An answer was obtained in the following way. First a close examination was made of all parts eviscerated and a record was kept of all organs showing variable structures. Special attention was given to Polian vesicles and to retracter muscles. The mutilated specimens were then placed in separate aquaria in which the water was changed frequently to prevent it from becoming stale. After a considerable interval these animals were killed and the regenerated organs were compared with the lost parts. Table IV shows several individuals compared in this way. The number of retractor muscles found in each radius is given in the order of the radii taken in a clockwise direction. A study of the table indicates that there is a strong tetndency to reproduce individual peculiarities, as shown by individuals *B, E, G, H, M* and *O*. This does

not always hold true, for individual *L* reverted toward the more radial type of symmetry. From these few cases it would appear that individual peculiarities tend to predominate over ancestral influences in the process of re-

TABLE IV

TO ILLUSTRATE THE RELATION BETWEEN REGENERATION AND ORIGINAL SYMMETRY IN THYONE

Individual Used	Original Symmetry		Regenerated Symmetry	
	Polian Vesicles	Retractor Muscles	Polian Vesicles	Retractor Muscles
B	2	2–2–2–2–2	2	3–3–2–3–3
E	2+	3–3–2–2–2	2	2–3–2–2–2
G	2	1–1–1–1–1	2	2–1–1–1–1
H	2	1–2–2–2–1	2+	2–2–2–2–2
L	1	1–1–1–1–1	2	2–3–2–2–2
M	1	1–1–1–1–1	?	1–2–1–1–2
O	3+	2–2–2–2–2	?	2–2–1–2–2
W	..	....	2	2–2–2–3–4
X	..	....	2	2–2–2–2–2
Y	..	....	2	2–2–1–2–2

generation. Specimens *W, X, Y,* are included in this table to show further the correlation between Polian vesicles and retractor muscles.

DISCUSSION AND SUMMARY

There remains to be discussed the general bearing of the foregoing experiments. First, the difference in the number of Polian vesicles in different Thyone is partly compensated by a variation in size, the fewer the number the larger their size, though this ratio would not be an exact one. In other words the total volume of the Polian vesicles in any given specimen bears a general relation to the size and functional activity of the animal. Notwithstanding this functional relationship since the actual number varies so widely it would be interesting to compare the number found in other species of holothuria with the conditions in Thyone. The data secured on this question were meager and not very definite. For example, Packard in one of the older text-books says in speaking of Thyone,

There are three Polian vesicles, one fusiform and an inch in length, the two others slenderer.

Clark ('02) gives the number for *Thyone briareus* (Leseur) as usually one or two; for *T. scabra* (Verrill) as usually single, and for *T. unisemita* (Stimpson) as one. He also mentions six other holothurians found in the Woods Hole region and all have a single Polian vesicle except *Cucumaria frondosa* (Gunnerus), which usually has one. He says nothing of the position in which these vesicles are found. In another paper ('01) Clark mentions a large holothurian about 40–45 centimeters in length (*Holothuria mexicana* Ludwig) in which there is a great diversity in the number of tentacles and Polian vesicles. The tentacles vary from 18 to 21, while the Polian vesicles vary from 1 to 9. The number of specimens examined, sixteen, was hardly sufficient to obtain an adequate comparison; two had 1 vesicle each, two had 2, five had 3, three had 7, one had 8, and one had 9. It is probable that if one were to examine a large number of individuals of each species, with reference to the number and location of the vesicles, he would obtain further interesting results. Lang ('96) cites a number of groups of holothurians in which only one vesicle has been observed; but states that there are a number of species in other groups that have occasionally or usually more than one.

Where accessory vesicles occur they vary greatly in number, and appear to have very slight, if any, systematic significance. Where only one Polian vesicle occurs it lies in the left ventral interradius, very seldom in the left dorsal interradius. Where two or more vesicles occur, they are also mostly formed in the ventral region of the circular canal.

Since Lang describes Cucumaria as the type specimen, in which the Polian vesicle is said to be in the left ventral region, it is possible that his generalizations were based principally on this form. At any rate, the conditions in Thyone seem to give a more definite significance to the number and location of the Polian vesicles.

Various explanations of autotomy and evisceration have been suggested, many of them having a teleological character. The view that the holothurian offers up the better

part of itself to appease the hunger of its enemy lacks confirmation, since the viscera are distasteful to fishes and to some other animals. It may be that the autotomous elimination of the Cuvierian organs serves a defensive purpose, as pointed out by Ludwig and Minchin, and Minchin suggests that the viscera may also be lost in this process and thus incidentally be associated with a protective response. In the case of Thyone, however, evisceration can hardly be considered defensive, and certainly it is not a process of self-division for only one part produces a new individual. Clark ('99) in discussing self-mutilation in the synaptas states the matter clearly in the following terms:

> I agree entirely with Cuenot ('91) in believing that autotomy is not normal or defensive but is due entirely to pathological conditions. I never saw a case of it in synaptas supplied with plenty of sand and an abundance of sea water.

Lang ('96) points out one of these pathological conditions, and recounts the fact that

> A Stichopus was observed to come entirely out of its skin, *i. e.*, the whole integument dissolved into slime, so that only the dermo-muscular tube enclosing the viscera remained.

In the present paper I have mentioned that Thyone at times appears to undergo a similar softening of the tissues in the region where the break occurs, and Pearse ('09) showed that autotomy is due, at least in part, to a structural arrangement which he considers is accidental in character. My observations further show that local constrictions undoubtedly have an important part in separating the retractors from the radial longitudinal muscles. All of these factors are pathological and are due to external or internal stimuli. The external (extra-cellular) stimuli, mechanical and chemical, as tried by Pearse ('08), appear to be less effective in producing autotomy than the purely internal (intracellular) stimuli such as lack of oxygen and its associated phenomena. The chemical (strychnine) that produced the largest percentage of evisceration in Pearse's experiments, probably affected respiration, since it greatly increased the activity of the

animal; therefore the need of oxygen would be proportionately greater than the supply, and the Thyone rendered more susceptible to evisceration. Now while autotomy undoubtedly enables the animal to maintain its existence for a considerable period on a smaller supply of oxygen, the times when this would become necessary in nature are probably rare, and it would be futile to speculate upon what evolution yet has in store for the process.

According to Lang, the retractor muscles of the oral region have been derived by the splitting up of the originally simple longitudinal muscles, and this specialization became more marked as the oral tentacles became more highly developed and required increasing protection. Species are to be found in the Dendrochirotæ in which the separation and branching off of retractors from the longitudinal muscles has not yet been perfected. In regeneration the retractor muscles of Thyone are derived in the same way, *i. e.,* by splitting off from the longitudinal muscles, and such progress is made that they are fairly well developed by the time the tentacles take up the function of feeding. The increasing sensitiveness and the later activity of the regenerating animal are presumably associated with the development of a new nervous system.

If we may regard the bilateral echinoderm larva as representing an early phylogenetic stage rather than a larval adaptation to a free-swimming existence, we will now discuss the symmetry of Thyone. As stated above, it is generally agreed that the radial arrangement of parts of the echinoderm body is due to a fixed stage in its ancestral history. Some holothurians and spantangoids, show in their ontogeny first a free stage, second a radial stage, and finally a bilateral adult. During the development of asteroids that have a fixed embryonic stage, the early bilateral symmetry is soon disarranged by the development of organs on the left side of the animal. For example, the left hydrocœle takes the form of an unclosed water-vascular rosette which grows around the esophagus to form the ring canal and its appendages, and its connection with the dorsal pore gives rise to the stone

canal. Excepting the echinoids and crinoids in which there is either no distinct Polian vesicle or else a simple glandular structure, those echinoderms that have retained the most distinctive type of radial structures have also as a rule, retained the most symmetrical arrangement of the Polian vesicles. Presumably these forms, the asteroids and ophiuroids, have quite recently abandoned the fixed stage, and each individual usually has four Polian vesicles and a stone canal, one in each interradius. Among most of the holothurians a secondary bilateral symmetry has become superimposed over the radial type, and it is reasonable to suppose that there was a time in the ancestral history of Thyone when the Polian vesicles were symmetrically and radially disposed, or else the animal quit its fixed habits before the radial symmetry of the vesicles was thoroughly established. In the one case we would have a regression, a sort of backward retracing of the steps of evolution, or, which seems more probable, the ancestors of Thyone began a free-living existence before the radial arrangement of the Polian vesicles had become complete. Also the fact that the embryology of the holothurian egg is probably much compressed and shows no trace of a fixed stage indicates that the corresponding ancestral stage was comparatively short, or, very remote. Since the modern habits of Thyone are bilateral, and since it is altogether improbable that such habits would produce the present arrangement of Polian vesicles, the position of these organs must be due to ancestral influence.

Now the Polian vesicles are capable of contracting and expanding and their function when they are well developed is to act as accessory reservoirs of the water-vascular fluid. Muscle and connective tissue in the wall of the vesicle furnish the means to do this work. Of course, if the ampullæ are well developed there is little or no need of Polian vesicles, as is the case in Asterias. But, though the size and number of these vesicles is functionally correlated with the general development of the water-vascular system, especially of the oral tentacles,

and hence shows great variability in the different species of holothurians, this does not in any way explain the great excess of these vesicles on the left side of *Thyone briareus*. In regeneration, probably through the influence of functional correlation, there is a tendency for the old tissue to reproduce the exact number and arrangement of the lost vesicles, but it may reproduce a somewhat more radial (ancestral) arrangement.

Enough has been given in this paper to show the need of a more extensive and intensive reexamination of the Polian vesicles. This would give a better idea of their morphological and functional significance. The following summary and conclusions are based on the work described:

1. Evisceration in Thyone includes the following organs: Esophagus, stomach, intestine, calcareous ring, nerve ring, tentacles, ring canal, Polian vesicles, stone canal with madreporite, and the retractor muscles of the esophagus.

2. The method used to produce evisceration was to allow Thyone to stand in stagnant water until it became foul. This was followed by treatment with running water containing much oxygen. Alternating these processes produced as high as 65 per cent. of self-mutilated individuals.

3. The structural accident theory of Pearse is inadequate to explain all of the conditions arising in the process of autotomy. At times the skin appears to dissolve away with little or no pressure present, and retractors frequently break off by local constrictions instead of by longitudinal pull.

4. The parts eviscerated are at first highly irritable, and may be kept alive for some time. The part remaining is less responsive, but reacts to touch, to lack of oxygen, and probably to other stimuli.

5. Regeneration of all lost organs may occur, but it takes place only when all parts concerned in evisceration are completely expelled. Otherwise the animal dies.

6. During the process of regeneration the behavior gradually becomes more responsive and finally is like the normal individual. This appears to be correlated with the growth of a new nervous system.

7. Thyone is functionally a bilateral animal, but the most conspicuous individual differences involve structures that have a radial arrangement.

8. The Polian vesicles vary greatly in number, size and location. There is a strong tendency for these to occur on the left side, and this arrangement is undoubtedly due to ancestràl conditions, for the present bilateral habits of Thyone could probably have no influence in producing this asymmetry.

9. The retractor muscles in a single radius consist of single or multiple strands, and this variation is closely correlated with a similar variation in the number of Polian vesicles. No explanation is forthcoming for this peculiar plasticity of the retractor muscles, but the suggestion is made that it may be functionally correlated with the development of the water-vascular system.

10. It was found from the study of a number of specimens that individual peculiarities of structure tend to be reproduced in the process of regeneration. In this process it would appear that individual variations tend to predominate over generalized ancestral influence.

11. Autotomy enables Thyone to survive for a considerable period on a smaller than normal supply of oxygen. Nevertheless, the conditions which give rise to self-mutilation are seemingly in all cases pathological.

12. The conditions in Thyone afford some evidence for believing that when this animal abandoned the fixed stage the Polian vesicles conformed more or less to the radial type. This is opposed to the statement of Lang that in all cases where a multiple number is now present "there was originally only one vesicle." It is believed that the present arrangement of Polian vesicles in Thyone can be best accounted for on the theory of phylogenetic influence. That, in general, those vesicles have retained their most complete radial arrangement in those species of

echinoderms which have maintained to a high degree the functional activity of the water-vascular system.

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